Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates

(ABSTRACT) Although the gene defect responsible for Huntington disease (HD) has recently been identified, the pathogenesis of the disease remains obscure. One potential mechanism is that the gene defect may lead to an impairment of energy metabolism followed by slow excitotoxic neuronal injury. In the present study we examined whether chronic administration of 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase, can replicate the neuropathologic and clinical features of HD in nonhuman primates. After 3-6 weeks of 3-NP administration, apomorphine treatment induced a significant increase in motor activity as compared with saline-treated controls. Animals showed both choreiform movements, as well as foot and limb dystonia, which are characteristic of HD. More prolonged 3-NP treatment in two additional primates resulted in spontaneous dystonia and dyskinesia accompanied by lesions in the caudate and putamen seen by magnetic resonance imaging. Histologic evaluation showed that there was a depletion of calbindin neurons, astroglialosis, sparing of NADPH-diaphorase neurons, and growth-related proliferative changes in dendrites of spiny neurons similar to changes in HD. The striosomal organization of the striatum and the nucleus accumbens were spared. These findings show that chronic administration of 3-NP to nonhuman primates can replicate many of the characteristic motor and histologic features of HD, further strengthening the possibility that a subtle impairment of energy metabolism may play a role in its pathogenesis.

Huntington disease (HD) is an inherited neurodegenerative disease characterized by choreiform movements, cognitive impairment, and emotional disturbance. Although the gene defect in HD has recently been identified, the mechanism by which it leads to neuronal degeneration remains obscure (1). A leading hypothesis is that excitotoxicity may contribute to the pathogenesis of HD (2, 3). Studies in both rodents and primates show striking similarities between striatal lesions produced by N-methyl-D-aspartate (NMDA) agonists and the neurochemical and histologic features of HD (4-6). Further evidence in support of an NMDA excitotoxic mechanism is the finding of a preferential loss of striatal NMDA receptors, which may occur early in the disease process (7, 8). One means by which slow excitotoxic neuronal death may occur is as a consequence of a defect in energy metabolism (2, 3). Disruption of ATP synthesis may lead to partial neuronal depolarization with activation of voltage-dependent NMDA receptors and secondary excitotoxic neuronal damage (9-12). Under these circumstances ambient levels of glutamate are sufficient to induce neuronal death. Several studies have reported decreased glucose metabolism and abnormalities in electron transport enzymes in HD (2). We recently obtained in vivo evidence of impaired oxidative metabolism in HD by demonstrating increasing concentrations of lactate in the basal ganglia and cerebral cortex using NMR spectroscopy (13). If a defect in energy metabolism plays a role in the pathogenesis of HD, it should be possible to model the neurochemical and histologic features of the illness with mitochondrial toxins. We recently found that chronic low-grade systemic administration of 3-nitropropionic acid (3-NP) to rats produces age-dependent lesions that closely mimic HD (14, 15). In the present study we examined whether administration of 3-NP to nonhuman primates could replicate both the movement disorder as well as the neuropathologic features of HD.

MATERIALS AND METHODS

We examined 11 nonhuman primates (three Macaca nemestrina and eight Papio anubis baboons) in this study. Two macaques and four baboons received 3-NP, whereas the remaining five animals served as controls for the behavioral studies and received saline. After completion of the behavioral study, the six 3-NP-treated primates (two macaques and four P. anubis baboons) were sacrificed for histological evaluation and compared with three of the control saline-treated primates (one macaque and two P. anubis baboons).

3-NP Treatment. 3-NP (Sigma) was dissolved in 5 M sodium hydroxide, pH 7.4, and then diluted to 80-180 mg/ml with deionized water. 3-NP was made fresh weekly. We initially examined a dose regimen of 3-NP at 12 mg/kg per day in one aged macaque; this dose was based on the toxic dose previously shown in rats to produce a progressive striatal degeneration (14). We administered two daily i.m. injections of 3-NP (6 mg/kg) during 5 consecutive days. Because severe behavioral alterations were observed in this acutely treated animal, a more progressive regimen of intoxication was used in three further aged animals (one macaque and two baboons) to produce a more chronic model of striatal degeneration. These three animals received 3-NP at a dose of 8 mg/kg daily, administered in two i.m. injections at 10 A.M. and 4 P.M. for 3-6 weeks. Two additional preadolescent P. anubis baboons were injected daily for 4 months at an initial dose of 10 mg/kg, which was progressively increased to 28 mg/kg.

Abbreviations: 3-NP, 3-nitropropionic acid; HD, Huntington disease; NMDA, N-methyl-D-aspartate.

*To whom reprint requests should be addressed at: Neurology Research/Warren 408, Massachusetts General Hospital, Boston, MA 02114.
Behavioral Testing. As shown previously for excitotoxic striatal lesions in primates, no spontaneous abnormal movements were initially observed in the chronically treated animals. However, when challenged with apomorphine (0.25–1 mg/kg i.m.) lesioned animals treated for at least 3–6 weeks showed typical choreiform movements and increased locomotor activity. Both before (pretreatment), during, and several times after the 3-NP treatment, behavioral alterations induced by apomorphine administration were videorecorded, coded, and analyzed blind post hoc by using two different techniques.

Quantitative videorecording analysis. Immediately after apomorphine administration (1 mg/kg), animals were placed in a large Plexiglass video cage (100 × 100 × 180 cm) and videorecorded using two different cameras located in front and on the top of the cage. Quantitative video image analysis was recorded for 40 min. The locomotor activity of the animals was evaluated from top-view images using a video-based motion tracking analysis system (16). With this analysis system, data were analyzed between 2 and 15 min after apomorphine injection. Alternate video frames were digitized, and a centroid was fitted to the position of the animal by subtracting each image from the image of the empty cage, smoothing the image, and bringing the image to threshold. The position of the animals’ center of mass was thus estimated, at 50-msec resolution, by determining the geometric center of each centroid. After low-band-pass filtering of the position data (cutoff = 3 Hz) all individual movements of the animal were extracted and characterized by their peak tangential velocity. The locomotor activity of each monkey for every session was then characterized by the average tangential velocity.

Clinical rating. A clinical rating scale was obtained as described after excitotoxic striatal lesions in baboons (17, 18). The incidence of four different categories of abnormal movements observed after apomorphine at 1 mg/kg i.m. (orofacial dyskinesia, dyskinesia of extremities, dystonia, and choreiform movements) was monitored and rated as being present (=1) or absent (=0) during each 5-min time period of the 40-min test session. The maximum incidence of each symptom was, therefore, a score of 8 during each test session. In addition, a summary score (sum of incidences) was computed by adding together the incidence of each symptom during the 40-min test period (maximum summary score = 32).

MRI Imaging. Animals were anesthetized with ketamine/xylazine, and MRI imaging was done on a 0.5-T MR magnet (General Electric) using a homemade Helmholt receive-only probe (19).

Histological Evaluation. Twelve to 15 weeks after termination of the 3-NP treatment, animals under chemical restraint (ketamine, 10 mg/kg) were euthanized by i.v. administration of pentobarbital (120 mg/kg). The brain was rapidly removed, cut in 2- to 3-cm-thick frontal slices, postfixed overnight in 4% (vol/vol) paraformaldehyde/sodium m-periodate/lysine, cryoprotected in phosphate buffer/15% (vol/vol) glycerol, and kept frozen at −70°C. Histochemical and immunohistochemical procedures were done as described (4, 6, 15). Frozen sections were cut at 50 µm and subsequently processed for Nissl staining, acetylcholinesterase, and NADPH-diaphorase histochemistry and calbindin DK28 immunohistochemistry. Golgi staining was done in one 3-NP-treated P. anubis baboon and compared with a control P. anubis baboon. Cell counts were made on Nissl/NADPH-diaphorase 50-µm-stained sections in 8–10 different areas (450 µm²) in the dorsal and ventral caudate nuclei of 3-NP-treated and control animals. Statistical analysis was made by using ANOVA. The results are expressed as the ratio of NADPH-diaphorase/Nissl neurons ± SEM.

RESULTS

In the aged macaque treated acutely with 3-NP, no symptoms were observed during the first 3 days of treatment. Starting at the fourth day, some episodes of self-biting and dystonia were observed, as well as a severe decrease in locomotor activity and bradykinesia (slowness of movements), rendering apomorphine testing impossible. After completion of 3-NP treatment, the animal showed motor symptoms, such as a marked loss in muscle tone and dystonic postures. MRI evaluation of this animal using a 0.5-T magnet (MR; General Electric) was done 2 days after termination of the 3-NP treatment (19). Transverse relaxation time (T2)-weighted images (TE/TR; 2000/25

![Fig. 1](https://example.com/image.png)

Clinical ratings of primates chronically treated with 3-NP as compared with control animals. Animals were injected with apomorphine (1 mg/kg) and videorecorded from the front for 40 min. Animals were rated for the incidence of four distinct classes of abnormal movements during each 5-min interval of a 40-min test session (11, 13). The maximal score of each symptom was, therefore, 8 during each test session. In addition, a summary score (sum of incidences) was computed by adding the maximal incidence for each of the four symptoms during the 40-min test session (maximum summary score is 32). Summary score is the sum of the incidence of all categories of abnormal movements. Black bars (CTRL) represent control animal (n = 5). Grey bars represent 3-NP-treated animals at different time points after termination of the 3-NP intoxication (1 day, 1, 2, 4, 6, and 8 weeks). Values are mean ± SEM for one session for controls and six sessions in the 3-NP-treated animals. Differences between controls and 3-NP-treated animals were tested by ANOVA.
values represent 3-NP-treated animals. In 3-NP-treated animals, apomorphine produced a significant increase in abnormal movements 1 week after treatment, which then persisted for up to 8 weeks (Fig. 1). The incidence of dystonia, dyskinesia of the extremities, and chorea-like episodes was increased by 4.1-, 3.8-, and 5.4-fold, respectively, as compared with control animals.

Quantification of locomotor activity at different time points after the 3-NP treatment using the video movement analysis system confirmed the clinical evaluation and showed a significant 3- to 5-fold increase in the average tangential velocity in 3-NP-treated animals after apomorphine treatment as compared with control animals (Fig. 2). Apomorphine (0.25-1.0 mg/kg, i.m.) induced a dose-dependent increase in locomotor activity and average tangential velocity without producing stereotyped movement (data not shown). Two younger baboons were treated initially with 3-NP at 10–18 mg/kg per day for 6 weeks, followed by 6–9 additional weeks of 3-NP treatment at 18–28 mg/kg per day. After 8 weeks of treatment, apomorphine (0.5 mg/kg) produced dyskinetic movements with a mean summary score of 18/32, which then increased to 26/32 after another 6–8 weeks of treatment. At 3 months of 3-NP treatment both animals showed spontaneous onset of foot dyskinesia and dystonia, which worsened in the presence of an observer. MRI showed decreased (increased) signal on longitudinal relaxation time ($T_1$) – $T_2$-weighted images in both the caudate and putamen (Fig. 3). Histologic evaluation confirmed the presence of selective striatal lesions (Fig. 3) with no damage in the thalamus and hippocampus.

Histopathologic evaluation of the three chronically treated aged animals (one M. nemestrina and two P. anubis) revealed bilateral lesions within the dorsal caudate nucleus and putamen, as compared with control animals (Fig. 4 A and B). Double-stained sections using Nissl stain and NADPH-diaphorase enzyme histochemistry revealed marked neuronal loss and gliosis within the striatum with relative sparing of medium-sized aspiny NADPH-diaphorase striatal neurons (Fig. 4 A, C, and E). There was a dorsoventral gradient of neuronal loss and gliosis with the dorsal caudate nucleus and putamen being most severely affected. Neuronal counts in Nissl/NADPH-diaphorase double-stained sections identified a significant ($P < 0.01$) 2.5-fold increase in the ratios of NADPH-diaphorase/Nissl neurons within the dorsal aspects of the striatum in 3-NP-treated animals as compared with control specimens (NADPH-diaphorase/Nissl ratio: lesion dorsal caudate nucleus, 6.2%; lesion ventral caudate nucleus, 3.2%; control dorsal caudate nucleus, 2.5%; control ventral

tongue protrusion. The incidence of apomorphine-induced licking behaviors in control animals was used as an endpoint for comparison with the orofacial dyskinesias observed in the 3-NP-treated animals. In 3-NP-treated animals, apomorphine produced a significant increase in abnormal movements 1 week after treatment, which then persisted for up to 8 weeks (Fig. 1). The incidence of dystonia, dyskinesia of the extremities, and chorea-like episodes was increased by 4.1-, 3.8-, and 5.4-fold, respectively, as compared with control animals.

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FIG. 2. Quantitative locomotor analysis of primates chronically treated with 3-NP as compared with control animals. Control and 3-NP-treated animals were injected with apomorphine (1 mg/kg) and videorecorded from the top for 40 min. Movements were automatically analyzed using a video-based motion tracking system (16), providing objective measurements of the average tangential velocity. The $y$ values represent average tangential velocity (ATV). Each dotted-square point is the mean ATV value from three animals determined during the first 10 min after apomorphine injection. The black bar (control, CTRL) represents control animals ($n = 3$). The gray bar (3-NP) represents 3-NP-treated animals recorded at 1 day and 1, 2, 4, 6, and 8 weeks after termination of treatments. Values are the mean ± SEM for one session in the control and six sessions in the 3-NP-treated group. Differences between the control group and 3-NP-treated group were tested by unpaired Student’s $t$ test.

ms) showed increased signal intensity in both the putamen and caudate nucleus. There were also slight changes in signal intensity in the hippocampus, consistent with observations in acutely treated rats (15). In the following weeks, despite a partial recovery, this animal could not sustain itself and required hand-feeding. The monkey was euthanized 3 weeks after the last 3-NP injection. Histological evaluation of this animal confirmed massive neuronal loss and gliosis within the putamen and caudate.

In the three animals treated with a chronic regimen of 3-NP (8 mg/kg per day for 3–6 weeks), no spontaneous abnormal movements were observed. However, after apomorphine testing (1 mg/kg, i.m.), various abnormal movements were observed, including dyskinetic and dystonic movements. Choreiform movements consisted of irregular and partial movements of the extremities and the trunk, resembling the dyskinesia and chorea observed in HD. Orofacial dyskinesia consisted of repetitive jaw movements accompanied by tongue protrusion. The most common forms of dystonia observed in the 3-NP-treated primates were twisting of the trunk and unilateral or occasionally bilateral foot dystonia. Apomorphine-induced abnormal movements in controls consisted of slow jaw movements, directed to some support (e.g., the glass walls), and repetitive licking behavior. After 3-NP treatment, jaw movements were asymmetrical and were always accompanied by

FIG. 3. Striatal damage in a baboon treated with 3-NP. (A) Calbindin-stained striatal section with marked damage to the caudate nucleus and putamen. There is relative preservation of the patch/matrix pattern in the ventral striatum consistent with sparing of calbindin-positive neurons in this area. (B) $T_2$ weighted image taken 2 days after completion of 3-NP treatment. Note the high signal intensity in the caudate nucleus and putamen corresponding to the damage seen in A.
caudate nucleus, 2.6%). Calbindin immunoreactivity, a marker for medium-sized spiny striatal neurons, was significantly reduced in 3-NP-treated animals (Fig. 4 B, D, and F). The number of calbindin-positive neurons was severely reduced within the dorsal striatum with relative sparing in the ventral striatum. Consistent with the pattern of calbindin neuronal loss, patch/matrix compartments identified with calbindin immunoreactivity were also affected. The patch/matrix pattern observed with acetylcholinesterase was spared (data not shown). Proliferative changes consisting of increased spine density, recurved distal dendritic segments, and growth cone-like structures on terminal segments of the dendritic arbor were present in medium-sized spiny striatal neurons or 3-NP-treated animals, as compared with controls (Fig. 4 G and H). Similar histopathologic alterations, however more pronounced, were present in the two baboons receiving prolonged 3-NP treatment.

**DISCUSSION**

In the present study we examined whether systemic administration of the known mitochondrial toxin 3-NP could produce both behavioral and histologic changes similar to those that occur in HD. 3-NP is a plant- and fungal-derived neurotoxin that is an irreversible inhibitor of succinate dehydrogenase (20). Ingestion of 3-NP has been linked to toxicity in both livestock and in humans. Exposure in human has occurred in China, where children ingested sugar cane contaminated with the fungus *Arthrinium* (21). The children developed an acute encephalopathy followed by appearance of a delayed-onset dystonia 11–60 days after ingestion. The patients show torsion spasms, torticollis, facial grimacing, and jerk-like movements. Computerized tomography scans show bilateral hypodensities in the putamen and, to a lesser extent, in the globus pallidus.

Initial studies of 3-NP toxicity in mice and rats showed that it produced striatal lesions accompanied by swollen mitochondria...
and a reduction of succinate dehydrogenase activity to 20% of control values (22, 23). We recently further characterized both the neurochemical and histologic features of 3-NP neurotoxicity in rats (14, 15). The lesions showed a striking age-dependence with young adult animals being much more vulnerable. The lesions were accompanied by reductions in ATP concentrations and focal increases in lactate confined to the basal ganglia as shown using MRI spectroscopy. Consistent with a slow excitotoxic mechanism, microdialysis studies showed no increases in extracellular glutamate concentrations, yet lesions were attenuated by prior decortication. This result is consistent with in vitro studies showing that 3-NP decreases cellular energy levels and acts as an indirect excitotoxin (24–26). We found that chronic low-grade administration of 3-NP for 1 month resulted in striatal lesions characterized by sparing of NADPH-diaphorase neurons and proliferative changes in the dendrites of spiny neurons similar to changes in HD (15).

In the present study we have extended these observations to primates. Studies in primates have the advantage that their motor repertoire much more closely resembles that of humans, and one can attempt to produce chorea, a cardinal manifestation of HD. Our prior work and that of others (6, 17, 18, 27) showed that dopamine agonists can produce chorea and dyskinesias in primates after excitotoxic lesions. The present study shows that chronic administration of 3-NP to primates results in an apomorphine-inducible movement disorder that closely resembles that seen in HD. The animals showed orofacial dyskinesia, dystonia, dyskinesia of the extremities, and choreiform movements. Both a clinical rating scale and a quantitative analysis of tangential velocity of individual movement velocities confirmed that the 3-NP-treated animals had a significant increase in hyperkinetic movements. Interestingly young baboons were resistant to 3-NP toxicity, requiring three times the daily dose used in adult animals to produce an apomorphine-inducible movement disorder. With prolonged administration of 3-NP in young baboons spontaneous abnormal movements developed, including foot dystonia and leg dyskinesia.

Histologic evaluation showed that the lesions were strikingly reminiscent of those that occur in HD. In HD there is a selective pattern of neuronal vulnerability with medium-sized spiny neurons, which contain calbindin, γ-aminobutyric acid, enkephalin, and substance P, being disproportionately affected early and most severely in the disease (28). In contrast, medium-sized aspiny neurons staining with NADPH-diaphorase and large cholinergic aspiny neurons are relatively spared (29, 30). After 3-NP administration, there was a marked depletion of calbindin spiny neurons with a relative sparing of both NADPH-diaphorase and large aspiny neurons. Recent studies in HD indicate that growth-related neuronal alterations in spiny striatal neurons precede degenerative changes (31). After 3-NP, Golgi studies showed proliferative changes in the dendrites of spiny neurons similar to changes in HD. The patch-matrix compartmentalization of the striatum, which is preserved in HD, was also spared by the lesions (28). Lastly the nucleus accumbens, which is spared in HD (32), was spared by the lesions.

These findings, therefore, show that chronic 3-NP administration in primates can produce both an apomorphine-inducible movement disorder and spontaneous abnormal movements with prolonged exposure, as well as histologic findings that replicate those of HD. They therefore strengthen the possibility that the gene defect may lead to an impairment of energy metabolism followed by slow excitotoxic neuronal death. If this is the case, then treatment with either excitatory amino acid antagonists, free radical scavengers, or agents to improve mitochondrial energy metabolism may prove useful in attempting to slow the degenerative process.

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